Background: Bone marrow (BM) derived stem cells secrete multiple growth factors and have been shown to differentiate to cardiomyocytes and endothelial cells in mice. This study evaluated the effect of trans-epicardial BM cell transplantation in a porcine non-reperfused myocardial infarction model.
Methods: In 14 domestic pigs, myocardial infarction was created by occluding the first diagonal artery with coils. Twenty-eight days later, Iliac crest BM cells were aspirated, filtered, labeled with bromodeoxyuridine (BrdU) and cultured for 48 hrs. Injections of 0.2 ml (~1.5 million cells/ml) (BM, n=11 pigs and saline, n=3 pigs) were evenly distributed 1cm apart in the scar (8 injections) and around the scar (8 injections). Animals were sacrificed at 4 (n=3), 14 (n=4) and 28 days (n=7) for histology and immunohistochemistry analysis. Trans-epicardial echocardiography was performed at the time of injection and at sacrifice to assess regional contractility.
Results: Positive BrdU cells were identified in infarcted areas at 4, 14 and 28 days of BM transplanted animals. Muscle cells (α-actinin positive cells) in the scar tissue were 21.1±15.7 /mm2 in the BM group and 13.6±2.4 /mm2 in the saline group at 28 days (p=0.07). At 28 days the number of endothelial cells (factor VIII positive cells) was greater in the BM group than in the saline group (19.5±8.9 vs. 8.9±15.1 cells/mm^2 p=0.07). Capillaries >50μm at 28 days were 10.95±3.02 /mm^2 in the BM group and 4.8±0.4 /mm^2 in the saline group (p=0.01). Wall Motion Score Index was 2.0±0.1 at baseline and 2.3±0.12 at 28 days in the BM group (p=0.18) and 2.0±0.1 in the saline group at 28 days, (p=0.40 vs BM).
Conclusion: Bone marrow cell engraftment is feasible with viable transplanted cells at 28 days within scar tissue of non-reperfused myocardial infarction. Angiogenesis was improved after BM injection. However, higher muscle cell area and vascular density in the scar were not correlated with improvement of left ventricular function at 28 days.

1178-140 Caldesmon Regulates Apoptosis and Cell Cycle Progression in Capillary Endothelial Cells and Inhibits Angiogenesis In Vitro

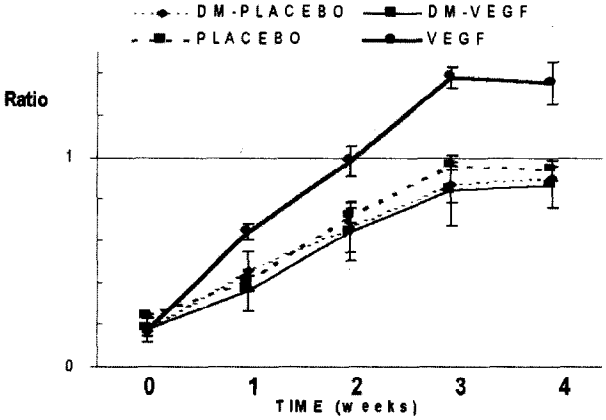
Yasushi Numaguchi, Sui Huang, Donald E. Ingber, Children's Hospital/Harvard Medical School, Boston, MA

Capillary endothelial (CE) cells can be switched between growth and apoptosis by modulating their shape and cytoskeleton (CSK) using micropatterned adhesive islands (Science 1997; 276:1425-1428). To further examine how the CSK contributes to this switching mechanism, we used an adenoviral vector carrying caldesmon, which inhibits CSK tension generation antagonizing Rho activity and disassembles acto-myosin filaments when overexpressed. Associated changes in cell shape and CSK organization were visualized in living cells by expressing GFP-caldesmon under tight control of a Tet-off system. Increase in cellular GFP-caldesmon resulted in progressive loss of actin stress fibers, disassembly of focal adhesions and cell retraction; the most highly retracted cells covered about one-third the project cell area of controls. The apoptotic index measured by quantitating TUNEL staining increased in parallel as GFP-caldesmon levels were increased and cell retraction was promoted. The smallest cells exhibited levels of apoptosis similar to that observed during anoikis in fully detached cells (42 vs. 55% TUNEL staining, respectively). Conversely, cell cycle progression into S phase (monitored by nuclear incorporation of BrdU at 24 hr) decreased from 35 to 9% as cells were progressively rounded up under similar conditions. In chicken aortic ring assay, GFP-caldesmon transfection inhibited vascular sprouting up to 60% if compared with those of GFP alone or control (5.2mm vs. 10.5mm vs. 12.1mm diameter of sprouting area, respectively). These data confirm that the CSK mediates the mechanism by which extracellular matrix-dependent changes in cell shape influence CE cell growth and apoptosis during angiogenesis. The adenoviral vectors encoding GFP-caldesmon under Tet-off control also may prove useful in future studies analyzing the role of CSK tension and structure during control of capillary development in angiogenesis.

1178-141 Can Vascular Endothelial Growth Factor Improve Blood Flow in Diabetes?

Ariel Roguin, Samy Nitecki, Irit Rubinstein, Edmond Sabo, Zaid A. Abassi, Nina S. Levy, Aaron Hoffman, Andrew P. Levy, Technion - IIT, Haifa, Israel, Rambam Medical Center, Haifa, Israel

Diabetes Mellitus (DM) is associated with increased cardiovascular morbidity and mortality. Collaterals can protect from ischemia. Considerable debate exists concerning the size and extent of the collateral circulation in DM. Our aim was to evaluate the usefulness of continuous perimuscular infiltration of naked-DNA encoding VEGF, to augment collateral formation and tissue perfusion in a DM mouse unilateral ischemic hindlimb model.
Methods: DM was induced with Streptozotocin (80 mg/kg). An osmotic pump with saline or 500-microgram VEGF was implanted intra-abdominaly with an outlet-tube fenestrated and tunneled into the muscle. Ischemic/normal limb blood flow was measured using a laser Doppler blood flowmeter once a week; tissues were analyzed for smooth muscle actin and factor-8. DM mice were compared to normal mice.
Results: In normal mice, a faster restoration of blood flow was observed in the VEGF treated, however in the DM mice, there was no difference in the rate of flow restoration between the VEGF-treated or placebo-treated arm (graph). The blood flow was almost complete for the normal mice but reached a ratio of 0.8-0.9 for both DM arms and for normal placebo. The blood vessel density was higher for the both DM arms compared to the normal mice.



Conclusion: Our results demonstrate the rapid and successful restoration of blood flow using naked-DNA encoding VEGF in a normal mice but lack of benefit of VEGF administration in DM mice. This may have major implication on angiogenic treatment in patients with DM.

1178-142 IL-6 Is Produced by Splenocytes Derived From CMV-Infected Mice in Response to CMV Antigens, and Induces MCP-1 Production by Endothelial Cells: A New Mechanistic Paradigm for Infection-Induced Atherogenesis

David Rott Jianhui Zhu, Mary Susan Burnett, Yi Fu Zhou, Alexandra Zalles-Ganley, Stephen E. Epstein, Washington Hospital Center, Washington, DC

Background: Atherosclerosis is an inflammatory disease. One of the candidate inflammatory triggers is infection. To further characterize the interaction between infection, cytokine induction, and atherosclerosis, we tested the hypothesis that cytomegalovirus (CMV) infection induces the pro-inflammatory cytokine interleukin-6 (IL-6), which in turn induces "pro-atherosclerotic" changes in vascular endothelial cells (ECs).
Methods: ELISA was used to determine the levels of monocyte chemoattractant protein-1 (MCP-1) in the supernatant of mouse and human ECs incubated with IL-6, and IL-6 levels in supernatants of splenocytes, derived from CMV infected and uninfected mice, stimulated with mouse CMV antigens.
Results: IL-6 induced, in a dose response fashion, MCP-1 expression in human ECs: 0, 2, 10, and 50 pg/ml IL-6 increased MCP-1 levels in EC conditioned medium from 1120±65, to 1148±105, 1395±40, and 2119±130 pg/ml, respectively, (P trend < 0.001). IL-6 also induced MCP-1 expression in mouse ECs (p<0.002). Importantly, IL-6 concentration in the supernatants of splenocytes stimulated with CMV antigens rose from undetectable levels in uninfected mice to14.9±5 pg/ml in the infected mice (P<0.04).
Conclusions: These results suggest a previously unrecognized, but potentially important mechanism whereby CMV, and other pathogens, contribute to atherogenesis: T-lymphocytes, clonally expanded in response to antigens presented by CMV infection, home to sites of vascular injury and locally release IL-6 when presented with either pathogen antigens that may be present in the plaque, or when they cross-react with host peptides homologous to the relevant pathogen antigens; IL-6 then triggers ECs to release MCP-1, which recruits more monocytes and T-cells into the vessel wall and thereby exacerbates local inflammation, and thus atherogenesis.

1178-143 Angiogenesis Induced by Human Hepatocyte Growth Factor Gene Without Nitric Oxide Synthesis in a Rat Ischemic Hindlimb Model

Makoto Ishizawa Koji Ohmori, Takahisa Noma, Teppei Tsuji, Kazushi Murakami, Tsunetatsu Namba, Katsufumi Mizushige, Ryuichi Morishita, Yasufumi Kaneda, Masakazu Kohno, Kagawa Medical University, Kagawa, Japan, Osaka University Medical School, Suita, Japan

Background: Vascular endothelial growth factor has been reported that it has the effect of angiogenesis through the nitric oxide (NO). Hepatocyte growth factor (HGF) has also been reported to have the effect of angiogenesis. However, it has not been reported whether HGF induces angiogenesis through the NO. In this study, we examined the feasibility of gene therapy using the HGF gene to treat peripheral arterial disease model rat in chronically inhibition of NO synthesis.
Methods: L-NAME (NO synthesis inhibitor) was obtained by drinking water (1mg/ml) from before resection of left femoral artery throughout this experiment. Sprague-Dawley rats were divided into three groups, which were transfected Human HGF naked plasmid DNA vector (500μg) or control vector (500μg) with L-NAME administration, and control vector without L-NAME administration. The naked plasmid was transfected into an ischemic hindlimb by intramuscular injection at1 week after resection. At 4 weeks after transfection, angiogenesis were assessed by angiography and tissue capillary density.
Results: At 4 weeks after transfection, blood pressure was significantly increased in rats administrated with L-NAME, but HGF transfection by intramuscular injection was not effect on blood pressure. The human HGF vector transfected rats administrated with L-NAME was resulted in significant increase in peripheral blood flow assessed by angiography compared with control vector administrated with L-NAME. Consistent with the increase in blood flow, a significant increase in tissue capillary number could be detected

in rats transfected with the HGF gene compared with control vector at 4 weeks after transfection.

Conclusion: Overall, those results may indicate that the effect of angiogenesis by local transfected HGF gene is not at least through the NO. Furthermore, transfected HGF gene may have the effect of angiogenesis in the several conditions of impaired NO synthesis.

1178-144 **In Vivo Electroporation of Hepatocyte Growth Factor Gene Into Skeletal Muscle of a Cardiomyopathic Hamster Ameliorates Cardiac Dysfunction and Fibrosis**

Yuko Tsujita, Kazuo Komamura, Masafumi Kitakaze, Kunio Miyatake, National Cardiovascular Center, Osaka, Japan

Background: Hepatocyte growth factor (HGF) has potent angiogenesis and antifibrosis effects. We examined whether the electroporation of HGF gene into skeletal muscle of dilated cardiomyopathy hamster could affect on cardiac function and fibrosis. **Methods** Plasmid vector expressing HGF (800 mcg) was transfected into the bilateral tibialis anterior muscles of 12 TO-2 hamsters of 11 weeks of age by electroporation once a week up to 14 weeks of age. Empty plasmid was transfected into other 12 hamsters. Echocardiographical, hemodynamic, histopathological and biochemical changes were measured before and after electroporation. **Results:** Electroporation increased the serum HGF levels to >10ng/mL in treated hamsters, whereas control hamsters showed no increase. LV ejection fraction (47.9+/-9.4 vs. 28.8+/-11.2 %, p<0.01), and E/A ratio (1.24+/-0.33 vs. 3.99+/-1.01%, p<0.05) were better in treated hamsters than in control hamsters. Systemic vascular resistance (3.31+/-1.30 vs. 7.34+/-4.99 mmHgmin/mL) was lower in treated hamsters than in control hamsters. Although left ventricular weight to tibialis length ratio (12.6+/-1.2 vs. 12.9+/-1.2 mg/g, NS) was similar, area of fibrosis in the ventricles (11.8+/-3.4 vs. 17.8+/-3.5 %, p<0.05) and hydroxyproline content (3.7+/-0.7 vs. 5.1+/-0.9 mmol/g, p<0.01) were less in treated hamsters than in control hamsters. Capillary density (1885+/-232 vs. 1447+/-182 vessel/mm2, p<0.01) was higher in treated hamsters than in control hamsters. **Conclusion:** These findings suggest that HGF gene transfer into muscle by electroporation is an effective means of delivery of HGF for treatment of heart failure due to dilated cardiomyopathy.

1178-145 **Transfection With DNA of Soluble VCAM-1 Causes Monocyte Chemotaxis and Increased Endothelial Cell Staining: A New Gene-Therapeutic Approach for Angiogenesis?**

Mirjam Decker, Uta Weirich, Thomas Nordt, Simone Bauer, Joerg Kreuzer, Christoph Bode, Karlheinz Peter, University of Freiburg, Freiburg, Germany

Background: Monocytes have been described as local mediators of angiogenesis. For monocyte recruitment, chemotaxis and adhesion to endothelial cells are major prerequisites. We hypothesized that soluble parts of endothelial adhesion molecules act as chemotactic factors and thereby may promote angiogenesis.

Methods and Results: Soluble (s) Adhesion molecules were tested for chemotactic activity using the monocytic cell line U937 in a 48-well microchemotaxis chamber. Only sVCAM-1, but not sICAM-1, or sE-selectin exhibited a significant concentration-dependent chemotactic effect at concentrations between 10 to 675nM. To test for a potential angiogenic effect of sVCAM-1, we designed an expression vector of a truncated form of the VCAM-1 gene encoding for sVCAM-1. After thoracotomy, rats were injected intramyocardially with naked sVCAM-1-DNA into the left ventricle. 21 days later, hearts were stained for macrophages with an ED1 antibody and for endothelial cells with indoxyl-tetrazolium (IT). Injection of sVCAM-1 DNA (n=10) results in 3.7±1.1% of ED1 positive area (area without injection (control, n=5): 0.03±0.02%, p<0.001) and in 8.5±2.2% of IT positive area (control: 5.1±0.9%, p<0.001). Injection controls with PBS (n=10), and the βGal gene (n=10) did not reveal statistically significant differences in macrophage and endothelial cell staining.

Conclusions: Transfection of soluble-VCAM-1-DNA induces chemotaxis on monocytes and causes an increase in endothelial cell staining in rat hearts. Further studies are warranted to prove that the transfer of soluble-VCAM-1-DNA represents a potential gene therapeutic approach for angiogenesis.

POSTER SESSION

1179 Vascular Mechanics

Tuesday, April 01, 2003, Noon-2:00 p.m.
McCormick Place, Hall A
Presentation Hour: 1:00 p.m.-2:00 p.m.

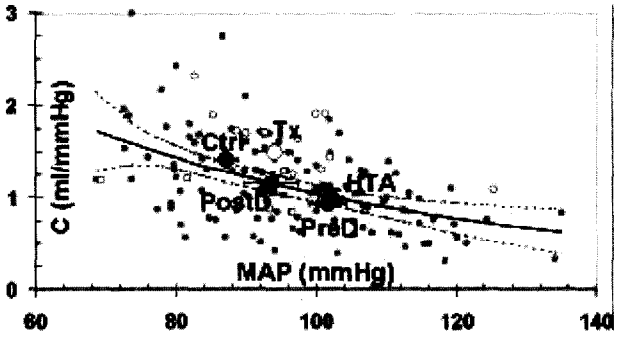
1179-146 **More Favorable Improvement of Arterial Wall Characteristics by Renal Transplantation Than by Hemodialysis**

Tine L. de Backer, Stephane G. Carlier, Brian A. Haluska, Patrick Segers, Han-Yo E. Ie, Thomas H. Marwick, Cardiovascular Center OLV Ziekenhuis, Aalst, Belgium, University of Queensland, Brisbane, Australia

Background: End stage renal disease is associated with reduced arterial compliance (C) and increased cardiovascular events. We evaluated C following hemodialysis (D) and renal transplantation (Tx).

Methods: Measurements were done in 32 controls(Ctrl, mean age 43y), 43 hypertensives(HT,58y), 18 D(58y) and 18 Tx pts matched to D for age and sex. Central pressure was derived by radial applanation tonometry using a transfer function. Aortic(Ao) flow was simultaneously measured with Doppler. C was evaluated by the pulse pressure method, an iterative search of the best fit between measured Ao pulse pressure and pulse pressure predicted by a 2-element Windkessel. In D pts recordings were done 1 hr before and 1 hr after dialysis.

Results: HT and preD had a significantly (p<0.05) higher mean arterial pressure (MAP) than Ctrl, postD and Tx. This led to significantly lower C for HT and preD (1.00±0.38 and 0.94±0.34), vs Ctrl, post D and Tx (1.42±0.54, 1.15±0.54, 1.49±0.40 ml/mmHg, respectively). After accounting for differences in MAP, Ctrl, HT, pre and post D values could all (•, graphic) be predicted by the same Langewouters' model(line and 95% CI). However, Tx(°) demonstrated supra-normal values of C for a MAP(94±11 mmHg) similar to postD but a pulse pressure (49±15 mmHg) similar to Ctrl.



Conclusion: Improvement of compliance after dialysis follows the non-linear Langewouters' pressure-volume relationship. Renal Tx improves arterial wall characteristics to a higher degree that may be related to structural changes.

1179-147 **Mental Stress Inhibits the Intimal Fibromuscular Proliferation Through Endogenous Opioid System in the Process of Arterial Remodeling**

Kunimitsu Iwai, Masayuki Matsumoto, Kenichi Kawanishi, Yukiharu Nishimura, Hiroshi Murai, Ling Y. Kong, Tsuyoshi Nakahashi, Hideyuki Hattori, Shigeto Morimoto, Kanazawa Medical University, Ishikawa-ken, Japan

Purpose Mental stress is speculated to be the trigger for the rupture of fibrous cap around the coronary arteriscleratic plaque. We investigated the influence of mental stress on the intimal fibromuscular proliferation in the rat model of arterial remodeling after endothelial injury in connection with two stress hormone systems. **Methos and Results** In Wister-Kyoto rats (eight groups,each:n=10) the endothelium of abdomial aorta was denuded with balloon catheter. (1)denudation (2)denudation + immobilization(6hrs/d) (3)denudation + naltolexone (NAL:2mg/kg/d ip) (4)denudation + NAL + immobilization (5)denudation + beta-endorphin(END:10ng/kg/d ip) (6)denudation + NAL + END (7) denudation + phentolamine(10ng/kg/d) + propranolol(10ng/kg/d) (8)denudation+ phentolamine+ propranolol + immobilization. The serum concentration of END was almost doubled (20pg/ml) by the immobilization stress. The area-ratio (R) of intima/media 14 days thereafter was examined. R was significantly reduced by immobilization (-62%:(2)vs(1)) and was completely restored by NAL (ns:(4)vs(1)). NAL itself had no significant influences (ns:(3)vs(1)). To the contrary, END reduced R (-70%:(5)vs(1)) and was also restored by NAL (ns:(6)vs(1)). Pharmacological blockade of sympathetic activity had little effects (ns:(7)vs(1)) and even under these blockades immobilization significantly reduced R (-59%:(8)vs(7)). The proliferating activity of the medial smooth muscle cells (SMC) assessed by PCNA immunohistochemistly 3 days after denudation showed the parallel results with the neointima formation. The migrating activity for the serum of medial SMC assessed by modified Boyden's chamber method in was also reduced by END (-26%) and completely restored with NAL. **Conclusion** These results indicate that mental stress stimulates the release of endogenous END, which inhibits the intimal fibromuscular proliferation by preventing both proliferation and migration of the medial SMC through opioid receptor. This mechanism may weaken the fibrous cap and make it easy to break.

1179-148 **Is a Radial-Aortic Transfer Function for Total Arterial Compliance Robust in Women and the Elderly?**

Brian A. Haluska, Leanne Short, Phillip Mottram, Stephane G. Carlier, Thomas H. Marwick, University of Queensland, Brisbane, Australia, OLV Hospital, Aalst, Belgium

Background: Estimates of central aortic pulse pressure for total arterial compliance (TAC) are based on a radial-aortic transfer function to calculate central pressure from radial applanation tonometry. However, this approach has been validated in groups with a preponderance of middle-aged men, and its validity in women and older patients has been questioned.

Methods: Carotid and radial applanation tonometry were performed simultaneously with pulsed wave Doppler of the LVOT using specialised software, in 96 pts (47 men; age 56±8y) with and without cardiovascular disease. TAC was calculated by the pulse-pressure method. Mean aortic pulse pressure (MAoP) was derived using a transfer function from radial tonometry, and then compared with the carotid waveform.

Results: The correlation between direct carotid measurement and radial measurement with the transfer function was good for TAC (r= .91). However, there was a significant difference in TAC in older patients (>65 years) using the two waveforms. Bland-Altman analysis of the difference (DIFF) between radial and carotid TAC showed significant differences between men and women (p=0.006) and between younger and older patients (p=0.05) (Table).